Role of lipids and lipoproteins in myocardial biology and in the development of heart failure

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ABSTRACT

As the population ages, heart failure will continue to be a growing public health problem. Metabolic homeostasis in the heart requires a fine-tuning of metabolism of different substrates. Notwithstanding a retro control of fatty acid and glucose utilization, the heart functions best when it oxidizes both substrates simultaneously. Mismatch between the uptake and oxidation of long-chain fatty acids in the myocardium induces lipotoxicity characterized by the accumulation of triglycerides, diacylglycerols, ceramides, and other lipids. Lipotoxicity may result in cardiomyocyte apoptosis, interstitial fibrosis, and cardiac dysfunction, and may promote insulin resistance. In this review, we will highlight the impact of lipids and lipoproteins on myocardial biology and on the development of heart failure independent of their effects on coronary heart disease.

KEY WORDS

Heart failure, cardiac metabolism, fatty acids, lipotoxicity, hypercholesterolemia, high-density lipoproteins

1. Introduction: epidemiological perspective of heart failure

Cardiovascular diseases remain the leading cause of mortality in Europe, causing 46% of all deaths[1]. These disorders cause a greater proportion of deaths among women (51%) than among men (42%)[1]. However, these deaths are more likely to occur in old age in women. The dramatic decline of the age-adjusted death rate for coronary heart disease and for stroke in the United States and in Europe[2] in the past 4 decades has led to a clear underestimation of the real impact of these diseases as reflected by crude mortality data.

The incidence and prevalence of heart failure are increasing[3]. The 5-year age-adjusted mortality rates after onset of heart failure are 50% in men and 46% in women[4]. Heart failure is the cardiovascular disease of this century in Europe and in the United States. As the population ages, heart failure will continue to be a growing public health problem. In this review, we will highlight the impact of lipids and lipoproteins on myocardial biology and on the development of heart failure independent of their effects on coronary heart disease. Since coronary heart disease indirectly influences the incidence of heart failure, we will briefly discuss prevention of coronary heart disease by dietary interventions in the next section with an emphasis on the controversial nature of dietary guidelines and on recent progress in this field.

2. Diet and prevention of coronary heart disease

The low-fat 'diet-heart hypothesis' has been the cornerstone of recommendations promulgated vigorously by the National Cholesterol Education Program, the National Institutes of Health, and the American Heart Association. This low-fat 'diet-heart hypothesis' dominated nutritional thought for several decades. However, these dietary recommendations have always been controversial given the lack of scientific evidence

and have come under intense attack for more than 10 years[5, 6]. The promotion of the low-fat diet was inspired by the classic Seven Countries Study that showed a direct correlation between dietary fat and total cholesterol levels and between total cholesterol levels and coronary-related mortality[5]. Surprisingly, the promotion of the low-fat diet began in earnest with the National Institutes of Health (NIH)-sponsored Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT), which demonstrated that cholestyramine resulted in a 19% reduction in risk ($p < 0.05$) of the primary end-point composed of coronary mortality and nonfatal myocardial infarction[7]. A leap of faith was made: if lowering serum cholesterol by medication was effective against coronary artery disease, reducing serum cholesterol by decreasing (saturated) fat intake would produce a similar result[6].

It should be stressed that the investigators of the Seven Countries Study did not claim causal relationships[8]. The relationship between diet and coronary heart disease is more complex. Serum cholesterol is not an intermediate variable that captures the complete effect of the diet. As a matter of fact, Ancel Keys, the lead investigator of the Seven Countries Study, observed in the early 1950s very low incidences of coronary heart disease in Naples and became a strong proponent of the 'good Mediterranean diet'[9]. The traditional Mediterranean diet was the diet found in olive growing areas of Crete, Greece, and Southern Italy in the late 1950s. Although there is heterogeneity in this diet in different geographic areas around the Mediterranean sea, the general characteristics can be summarized as follows: 1) a high consumption of cereals, legumes, nuts, vegetables, and fruits; 2) a relatively high-fat consumption, mostly provided by olive oil; 3) moderate to high fish consumption; 4) poultry and dairy products consumed in moderate to small amounts; 5) low consumption of red meats, and meat products; and 6) moderate alcohol intake, usually in the form of red

wine. In 1996, a protective effect of the Mediterranean diet was demonstrated in the Lyon Diet Heart Study[10]. The Lyon Diet Heart Study was a secondary prevention trial testing the protective effects of a Mediterranean type of diet supplemented with rapeseed oil and margarine (a source of α-linolenic acid (C18:3, *n*−3)) to replace butter and cream in the experimental group.

In 2013, the results of the landmark primary prevention PREDIMED trial were published[11]. A total of 7447 participants with no cardiovascular disease at enrollment were randomized to one of three diets: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet (advice to reduce dietary fat). The primary endpoint was the rate of major cardiovascular events (myocardial infarction, stroke, or death from cardiovascular causes). On the basis of the results of an interim analysis, the trial was stopped after a median follow-up of 4.8 years. The multivariable-adjusted hazard ratios were 0.70 (95% confidence interval [CI], 0.54 to 0.92) and 0.72 (95% CI, 0.54 to 0.96) for the group assigned to the Mediterranean diet with extra-virgin olive oil and for the group assigned to the Mediterranean diet with nuts, respectively, versus the control group. The Lyon Diet Heart Study in a secondary prevention setting and the PREDIMED study in a primary prevention setting provide strong evidence that the Mediterranean diet has become the standard for prevention of coronary events and for healthy eating. These studies, together with the important epidemiological data of Trichopoulou *et al*.[12], confirm the importance of the whole dietary pattern approach to preventive nutrition. A healthy diet should be thought of as a whole rather than as a recitation of good and bad components. In other words, the interactions between various nutrients is the real biological source of protection rather than any small change in specific nutrients[13]. This principle should also be kept in mind when studying the relation

between lipids and heart failure: a reductionist approach is hypothesis generating but does not provide any final answer.

3. Lipids as fuel for the heart

The stroke work is the work done by the ventricle to eject the stroke volume and corresponds to the area within the pressure-volume loop. Since the heart is continuously producing external work by ejecting blood into the aorta, the energetic requirements of this organ are well beyond the normal maintenance of cellular integrity[14]. Cardiac energy conversion is highly specialized to maintain physiological concentrations of energy-rich phosphate bonds under conditions of large variations of external work. The maintenance of cellular metabolites during alterations in workload has been termed metabolic homeostasis[14]. The heart has a relatively low ATP content (5 μmol per g wet weight) and a high rate of ATP hydrolysis (approximately 30 μmol per g wet weight per minute at rest)[15-17]. Hence, there is complete turnover of the myocardial ATP pool approximately every 10 seconds under normal conditions. The daily turnover of ATP in the human heart (13 mol or more than 6 kg) is many times higher than the weight of the heart[18]. Metabolic homeostasis requires a fine-tuning of cardiac metabolism of different substrates. Except in the postprandial period, the heart predominantly metabolizes free fatty acids (up to 70%). Notwithstanding a retro control of fatty acid and glucose utilization, the heart functions best when it oxidizes both substrates simultaneously[19]. Other competing energy substrates are lactate, ketones, and amino acids. A schematic overview of fatty acid ß-oxidation in cardiomyocytes and its reciprocal relationship with glucose metabolism is shown in Figure 1.

Fatty acids are supplied to the heart as either free fatty acids bound to albumin or as fatty acids released from triglycerides contained in chylomicrons or very-low-density lipoproteins (VLDL)[15]. The majority of lipids entering cardiac cells are diverted toward fatty acid utilization[20] with some being stored or used for structural requirements. The intramyocardial content of triglycerides in healthy subjects (approximately 3 mg per g tissue) is very low compared to the rate of free fatty acid uptake (approximately 3 mg per g tissue per hour)[15]. If 20% of the cardiac free fatty acid uptake enters the intramyocardial triglyceride pool[21], the mean turnover time for intramyocardial triglycerides is only 5 hours.

Normal circulating free fatty acid concentrations range between 0.2 mM and 0.6 mM[22]. Activation of the sympathetic nervous system, e.g. during effort or during fasting, results in increased circulating free fatty acid concentrations primarily resulting from β-adrenoreceptor-mediated stimulation of hormone-sensitive lipase activity in the adipose tissue[23]. Chronically elevated circulating free fatty acid concentrations occur in obese, insulin resistant subjects with dysregulated lipolysis and in patients with overt diabetes. The arterial fatty acid concentration is the primary determinant of the rate of myocardial fatty acid uptake and oxidation[20]. The majority of fatty acids used by the heart that originate from exogenous triglycerides are derived from chylomicrons and only a minor portion originates from VLDL[24]. Triglycerides within chylomicrons and VLDL require hydrolysis by lipoprotein lipase on the luminal surface of endothelial cells[25]. Lipoprotein lipase is associated with proteoglycans and glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GPIHBP1). Free fatty acids originating from either albumin or generated by the action of lipoprotein lipase in the capillary endothelium enter the cardiomyocyte either by passive diffusion or via protein carrier-mediated

pathways[25]. Uptake of whole triglyceride-rich lipoproteins in the heart may occur via the VLDL receptor[26]. Myocardial fatty acids can also be derived from *de novo* lipogenesis[25]. Deletion of fatty acid synthetase in heart of mice results in cardiac decompensation in the setting of aortic constriction or aging[27].

The Randle cycle, also known as the glucose fatty-acid cycle, describes the reciprocal relationship between fatty acid and glucose metabolism[28]. Pyruvate decarboxylation is the key irreversible step in carbohydrate oxidation and is catalyzed by pyruvate dehydrogenase, which is located in the mitochondrial matrix. Increased generation of acetyl coenzyme A (acetyl CoA) derived from fatty acid β-oxidation decreases pyruvate dehydrogenase activity via the activation of pyruvate dehydrogenase kinase and the subsequent phosphorylation and inhibition of pyruvate dehydrogenase. Both an increased acetyl CoA/CoA ratio and an increased $NADH/NAD^+$ ratio result in activation of pyruvate dehydrogenase kinase. Increased fatty acid β-oxidation also decreases glycolysis via inhibition of phosphofructokinase isoforms 1 and 2 mediated via increased cytosolic citrate concentrations. On the other hand, increased glucose oxidation will result in inhibition of fatty acid oxidation. Acetyl CoA transferred from the mitochondria to the cytosol is a substrate for acetyl CoA carboxylase, which generates malonyl CoA. Malonyl CoA inhibits carnitine palmitoyltransferase I, which is the rate-limiting enzyme in the carnitine palmitoyltransferase system. This system allows transfer of fatty acids to the mitochondria for ß-oxidation.

As in any mechanical pump, only part of the energy invested by the heart is converted to external power[29]. The mechanical efficiency is defined as the ratio of useful energy produced (stroke work) to oxygen consumed[30]. Invasive studies in healthy control subjects have demonstrated that approximately 25% of consumed oxygen is

finally converted to external work[30]. The residual energy is predominantly converted to heat and partially used for non-mechanical activity of basal metabolism and for excitation-contraction coupling[31, 32]. To generate the same external power, myocardial oxygen consumption is lower when the myocardium oxidizes glucose and lactate than when the myocardium is fuelled by fatty acid β-oxidation. When glucose and palmitate oxidation are compared, the complete oxidation of 1 palmitate molecule generates 105 molecules of ATP and consumes 46 atoms of oxygen, whereas the complete oxidation of 1 molecule of glucose generates 31 molecules of ATP and consumes 12 atoms of oxygen[15, 33]. Therefore, the phosphate/oxygen ratio of oxidative phosphorylation (the ratio of the number of molecules of ATP produced per atom of oxygen reduced by the mitochondrial electron transport chain) is higher for glucose than for palmitate. However, increasing the rate of fatty acid uptake of the heart by elevating plasma free fatty acid concentrations with an infusion of heparin and triglyceride emulsion increases myocardial oxygen consumption by approximately 25% without changing the mechanical power of the left ventricle[34, 35]. This difference is larger than the difference of the phosphate/oxygen ratio, which may be explained by increased uncoupling of mitochondrial oxidative phosphorylation and greater futile cycling during fatty acid oxidation[15].

4. Cardiac lipotoxicity

Mismatch between the uptake and oxidation of long-chain fatty acids in the myocardium induces lipotoxicity characterized by the accumulation of triglycerides, diacylglycerols, ceramides, and other lipids. Myocardial lipotoxicity may result in cardiomyocyte apoptosis, interstitial fibrosis, and cardiac dysfunction, and may promote insulin resistance[36]. Heart failure is frequently accompanied by

cardiomyocyte lipid accumulation and a clear correlation between increased myocardial triglyceride content and decreased cardiac function has been established in rodents and humans. The accumulation of intramyocardial lipids in dysfunctional and failing hearts led to the hypothesis that toxic lipids are mediators of cardiac dysfunction. The turnover of triglycerides in the heart is very rapid (approximately 5 hours) compared to adipose tissue (200-270 days)[37, 38]. Consequently, cardiac triglyceride accumulation is not inert but reflects a metabolic perpetuation of an imbalance between fatty acid uptake and oxidation. Triglycerides are unlikely the cause of the deterioration of cardiac function. Mice transgenic for diacylglycerol acyltransferase1 in the heart display normal cardiac function despite increased accumulation of triglycerides in the myocardium[39]. In contrast, accumulation of ceramides and diacylglycerols alter intracellular signaling pathways and promote apoptotic cell death. Ceramides can trigger apoptosis by inducing the release of cytochrome c from the mitochondria[40]. Diacylglycerols may interfere with the cardiac insulin-signaling cascade by activating protein kinase C, leading to decreased glucose uptake[41].

Cardiac dysfunction in obese and diabetic animals and in humans is unambiguously associated with prominent lipotoxicity[42]. The metabolic alterations associated with diabetic cardiomyopathy include excessive rates of myocardial fatty acid uptake and fatty acid oxidation coupled with reduced glucose utilization. In patients with insufficiently controlled type 1 diabetes mellitus, the heart is glucose-starved due a lack of insulin action and is forced to oxidize fatty acids as an alternative substrate to maintain cellular ATP levels. On the other hand, the type 2 diabetic heart is flooded with fatty acids, leading to inhibition of glucose oxidation in the mitochondria[42]. In both types of diabetes, metabolic flexibility is lost. Increased free fatty acids in

cardiomyocytes may activate the peroxisome proliferator-activated receptor α (PPARα) pathway. Ligand activation of the transcription factor PPARα increases cellular utilization of fatty acids by up-regulating a subset of genes that promote fatty acid uptake and genes that increase fatty acid ß-oxidation[43]. PPARα increases pyruvate dehydrogenase kinase 4 expression, which together with negative feedback of fatty acid oxidation reaction products decreases glucose oxidation[44].

Cardiac steatosis and reduced myocardial function are observed in mouse models of increased fatty acid utilization induced by cardiac-restricted transgenic expression of long-chain acyl CoA synthetase 1[45], fatty acid transport protein 1[46], or PPARα[47]. In clear opposition, myocardial overexpression of adipose triglyceride lipase leads to reduced fatty acid oxidation, is accompanied by reduced triglycerides stores and increased insulin sensitivity and glucose oxidation, and protects against pressure overload-induced cardiomyopathy[48]. In a murine model of streptozotocin-induced diabetes, cardiomyocyte-specific adipose triglyceride lipase expression protected against the development of diabetic cardiomyopathy[49]. Diabetes-induced increases in myocardial triglycerides, lipotoxic intermediate accumulation (ceramides, diacylglycerols, palmitoyl coenzyme A), and cardiac dysfunction were inhibited in mice with adipose triglyceride lipase expression under control of the myosin heavy chain promoter. Furthermore, these mice exhibited decreased reliance on palmitate oxidation and blunted PPARα activation[49].

The importance of fine-tuning of lipid homeostasis in the myocardium is underscored by the capacity of cardiomyocytes to export triglycerides via secretion of spherical lipoproteins[50]. In agreement, the heart expresses the two lipoprotein-assembly proteins that are mandatory for bulk secretion of triglycerides i.e. microsomal triglyceride transfer protein and apolipoprotein (apo) B. Transgenic mice

overexpressing a full length human *apo B* transgene are protected against obesity-induced cardiac steatosis and lipotoxic heart disease[51], which clearly supports a protective role of lipoprotein secretion in the myocardium. Apo B expression in the myocardium also prevents cardiac dysfunction in diabetic mice[52]. Similarly, apo B production in the myocardium attenuates lipotoxic heart disease induced by free fatty acid oversupply secondary to lipoprotein lipase overexpression in the heart[53].

5. Impact of fatty acids on heart failure: experimental animal studies and clinical data

The data presented in this section reflect a reductionist approach and should be interpreted cautiously. As already stated, a healthy diet should be thought of as a whole and interactions between various nutrients is very often the real biological source of protection rather than any small change in specific nutrients[13].

5.1. Saturated fatty acids

Higher plasma saturated fatty acids, especially palmitic acid (C16:0) and myristic acid (C14:0), were independently associated with incident heart failure in both men and women in the Atherosclerosis Risk in Communities Study[54]. Saturated fatty acids may directly promote the development of heart failure by inducing lipotoxicity[55]. However, most experimental animal studies on the role of saturated fatty acids in cardiac dysfunction have been based on obesogenic high-fat diets. Obesity-associated inflammation and systemic insulin resistance confound experimental investigations designed to study the causal role of saturated fatty acids in cardiac dysfunction and heart failure. The myristate-containing ceramide species C_{14}-ceramide has been implicated in the pathogenesis of lipotoxic cardiomyopathy in a milk-fat based diet

model that was associated with an approximately 20% increase of body weight[55]. A cocoa butter derived high-fat diet (containing mainly palmitate (C16:0) and stearate (C18:0)) did not have major effects on cardiac structure and function compared to a low fat diet in sham-operated mice and in mice with pressure overload induced by transverse aortic constriction[56, 57]. This cocoa butter derived high-fat diet resulted in minor effects[56] or no effects[57] on body weight. Taken together, many experimental studies on the effect of dietary saturated fatty acids are confounded by changes in body weight. A direct causal role of saturated fatty acids in cardiac dysfunction and heart failure is essentially unproven.

5.2. Monounsaturated fatty acids

Feeding experiments in rodents, pigs, and non-human primates published between 1960 and 1986 suggested that consumption of erucic acid (C22:1, *n*−9) and cetoleic acid (C22:1, *n*−11) resulted in cardiac steatosis and fibrosis[58-61]. Long-chain monounsaturated fatty acids (C20:1, C22:1, C24:1) are predominantly oxidized in peroxisomes rather than in mitochondria, which lack membrane-transporting enzymes for long-chain fatty acids[62, 63]. Fatty acid oxidation in peroxisomes produces reactive oxygen species and various cytosolic lipid metabolites that can cause cardiotoxicity and impaired myocardial function[15, 25, 62].

Erucic acid comprises 30-60% of fatty acids in unmodified rapeseed oil. The evidence from animal feeding studies on the potential cardiotoxicity of long-chain monounsaturated fatty acids[58-61] led Canadian farmers to develop Canola[63]. Canola is a modified rapeseed oil and is the acronym of CANadian Oil Low in erucic Acid. However, the potential toxicity of long-chain monounsaturated fatty acids was largely forgotten in recent decades till Imamura *et al*.[63] investigated the association

between long-chain monounsaturated fatty acids and the incidence of congestive heart failure in two prospective cohorts. After multivariable adjustment, higher plasma phospholipid levels of C22:1 and C24:1 but not C20:1 were associated with greater congestive heart failure incidence in both the Cardiovascular Health Study and in the Atherosclerosis Risk in Communities Study Minnesota subcohort[63]. These associations existed for ischemic congestive heart failure, valvular congestive heart failure, and non-ischemic non-valvular ischemic heart failure.

Sources of long-chain monounsaturated fatty acids include generally more healthy foods (fish, mustard seeds and oil, salad oils, and poultry) and less healthy foods (processed meats and mixed meals e.g. pizza and meat sandwiches). Since fish intake has been inversely associated with the incidence of congestive heart failure[64], the potential benefits of long-chain omega-3 polyunsaturated fatty acids likely outweigh any potential harmful effects of long-chain monounsaturated fatty acids.

5.3. Omega-3 polyunsaturated fatty acids

A meta-analysis of 7 prospective studies with a total of 176 441 subjects and 5 480 incident cases of heart failure concluded that fish intake and intake of marine omega-3 fatty acids (eicosapentaenoic acid (EPA) (C20:5, *n*−3) and docosahexaenoic acid (DHA) (C22:6, *n*−3)), lowered the risk of heart failure[65]. Plasma phospholipid levels of α-linolenic acid (C18:3, *n*−3) and dietary intake of α-linolenic acid were not associated with incident congestive heart failure in the Cardiovascular Health Study, a prospective cohort study of adults aged 65 years or older in the USA[66].

5.4. Omega-6 polyunsaturated fatty acids

After adjustment for age and other confounders, an inverse relationship was observed between plasma phospholipid arachidonic acid (C20:4, *n*−6) levels and congestive heart failure incidence in women but not in men in the Atherosclerosis Risk in Communities Study Minnesota subcohort[54]. An inverse relationship between plasma phospholipid linoleic acid (C18:2, *n*−6) levels and congestive heart failure incidence was observed in a model adjusted for age and sex[54]. In contrast, phospholipid dihomo γ-linolenic acid (C20:3, *n*−6) levels were positively associated with incident heart failure in a model adjusted for age and sex[54]. However, after adjustment for other confounders, the associations of linoleic acid levels and dihomo γ-linolenic acid levels with incident heart failure were attenuated. In a nested case-control analysis of the Physicians' Health Study, no association between total plasma omega-6 polyunsaturated fatty acids and risk of developing heart failure was observed in male US physicians[67].

6. Impact of lipoprotein levels on heart failure: experimental animal studies and clinical data

Epidemiological studies support a strong association between metabolic cardiovascular risk factors and heart failure incidence. The risk for incident heart failure in the Framingham Heart Study was 2.4-fold higher in diabetic men and 5.0-fold higher in diabetic women independent of age, coronary disease, hypertension, and body mass index[68]. In Framingham Heart Study participants free of coronary heart disease at baseline, low high-density lipoprotein (HDL) cholesterol and high non-HDL cholesterol were independently associated with heart failure incidence after adjustment for interim myocardial infarction and clinical covariates[69]. However, plasma non-HDL cholesterol levels strongly correlate with the intake of saturated

fat[70], which may confound the association between non-HDL and heart failure incidence. As stated in section 5.1, higher plasma saturated fatty acids, especially palmitic acid (C16:0) and myristic acid (C14:0), were independently associated with incident heart failure in both men and women in the Atherosclerosis Risk in Communities Study[54].

6.1. Non-HDL lipoproteins

Based on echocardiographic data, an early cardiomyopathy characterized by systolic and diastolic dysfunction has been described in patients with primary hypercholesterolemia without evidence of coronary heart disease[71]. Experimental rabbit data are in agreement with the hypothesis that hypercholesterolemia has direct effects on the myocardium. Hypercholesterolemia in rabbits induces electrical remodelling of the heart, characterized by prolongation of the action potential and of the heart rate corrected QT interval, increased repolarization dispersion, and vulnerability to ventricular fibrillation[72]. Using tissue Doppler imaging, systolic and diastolic dysfunction has been demonstrated in hypercholesterolemic rabbits[73]. Data on the effect of cholesterol on *in vivo* systolic and diastolic function are paralleled by observations in isolated cardiomyocytes showing a decrease in the maximum rate of cardiomyocyte shortening and the maximum rate of cardiomyocyte relaxation[74, 75]. Based on these observations, the term ‘cholesterol cardiomyopathy’ has been introduced[74]. However, these rabbit studies should be interpreted with caution. Indeed, plasma cholesterol levels in these studies were approximately 500 mg/dl[73] and 800 mg/dl[74], which is rarely observed in humans. Taken together, none of these experimental animal intervention studies have

investigated the impact of a pathophysiologically relevant degree of hypercholesterolemia on the development of heart failure.

Van Craeyveld *et al*.[76] have demonstrated that lipid lowering gene transfer in hypercholesterolemic mice results in reduced infarct expansion, decreased ventricular remodelling, and improved left ventricular contractility and relaxation post-myocardial infarction. This study was performed in a model of permanent ligation of the left anterior descending coronary artery. The interpretation of the experimental results in this model should take into account the effect of lipid lowering on infarct healing. Since lipid lowering improved infarct healing and attenuated infarct expansion[76], direct effects of lipid lowering on the myocardium cannot be unambiguously proven in this model.

As stated *supra*, high non-HDL cholesterol levels were independently associated with heart failure incidence after adjustment for interim myocardial infarction and clinical covariates in Framingham Heart Study participants free of coronary heart disease at baseline[69]. These prospective data are in agreement with earlier cross-sectional studies showing that post-infarct ejection fraction is lower in patients with elevated non-HDL cholesterol levels[77, 78]. Furthermore, mortality rates in patients developing congestive heart failure were 20% lower with simvastatin treatment compared with placebo in the Scandinavian Simvastatin Survival Study (4S)[79] but these data are observational. In contrast, data from randomized clinical trials evaluating the effects of statins in heart failure patients seem to contradict a role of non-HDL lipoproteins. In the CORONA randomized clinical trial[80], a total of 5011 patients at least 60 years of age with New York Heart Association class II, III, or IV ischemic, systolic heart failure were randomly assigned to receive 10 mg of rosuvastatin or placebo per day. The primary outcome (death from cardiovascular

causes, nonfatal myocardial infarction, or nonfatal stroke) occurred in 692 patients in the rosuvastatin group and 732 in the placebo group (hazard ratio, 0.92; 95% confidence interval, 0.83 to 1.02; P=0.12) during a median follow-up of 32.8 months. In a pre-specified secondary analysis, there were fewer hospitalizations for cardiovascular causes in the rosuvastatin group (2193) than in the placebo group (2564) (P<0.001)[80]. In the GISSI-HF trial, rosuvastatin 10 mg daily did not affect clinical outcomes in patients with chronic heart failure of any cause[81]. At least two factors may explain the apparent discrepancy between the results of these critically important trials on the one hand and the epidemiological observations and experimental animal studies on the other hand. Firstly, lipid lowering before the onset of overt heart failure may be critical to observe beneficial effects. Secondly, the precise biochemical effects of statins should be considered. Statins inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which converts 3-hydroxy-3-methylglutaryl-CoA to mevalonate. However, cholesterol biosynthesis is only one of the functions of the mevalonate pathway. Blocking the mevalonate pathway by statins also inhibits the synthesis of isopentenyl pyrophosphate. Isopentenyl pyrophosphate is a unique intermediate of the mevalonate pathway. This molecule is not only a building block for cholesterol synthesis but is also is required for the post-transcriptional enzymatic isopentenylation of selenocysteine tRNA and its maturation to a functional tRNA molecule. Inhibition of isopentenyl pyrophosphate synthesis by statins results in a decrease in available selenoproteins[82]. Among the 25 selenoproteins in mammals, the family of glutathione peroxidases is a major group. Each of these enzymes reduces lipid and hydrogen peroxides to lipid alcohols and water, respectively, and does so while oxidizing glutathione to glutathione

disulfide[83]. A reduction of myocardial glutathione peroxidases may impair cardiac function via increased oxidative stress[83].

Farnesyl pyrophosphate is a further downstream intermediate of the mevalonate pathway and is a precursor for cholesterol. The farnesyl moiety from farnesyl pyrophosphate is utilized for post-translational modification of proteins including small GTPases. Farnesyl pyrophosphate is also a precursor for geranylgeranyl pyrophosphate, which is similarly involved in post-translational modification of proteins. Furthermore, farnesyl pyrophosphate is a precursor of dolichol, heme-A, and ubiquinone (coenzyme Q10). Thus, endogenous coenzyme Q10 synthesis is blocked by 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins)[84]. Coenzyme Q10 is a component of the electron transport chain in the mitochondria and participates in aerobic cellular respiration leading to the production of ATP. Low plasma coenzyme Q10 levels are an independent risk factor for worsened outcomes in heart failure[85]. In a small recent randomised trial enrolling 420 patients, treatment with coenzyme Q10 in chronic heart failure patients reduced major adverse cardiovascular events[86]. Taken together, beneficial effects of cholesterol lowering on cardiac function induced by statins may be partially offset by the statin-induced reduction of coenzyme Q10 and by decreased myocardial glutathione peroxidase activity secondary to the statin-induced decline of isopentenylation of selenocysteine tRNA.

The topic of the side effects of statins is important and a source of significant controversies. One area of particular interest concerning the relation between serum cholesterol, statin use, and heart failure is the effect of statins on diabetes incidence. Using data from 20 randomised controlled trials, Swerdlow *et al*.[87] recently showed that statin treatment increased the risk of incident type 2 diabetes, with an odds ratio

(OR) of 1.12 (95% CI 1.06–1.18) versus controls, which confirms previous reports. Since the risk for incident heart failure in the Framingham Heart Study was 2.4-fold higher in diabetic men and 5.0-fold higher in diabetic women independent of age, coronary disease, hypertension, and body mass index[68], increased incidence of diabetes on statins may counteract potential beneficial effects of cholesterol lowering on cardiac function. Using a mendelian randomization approach based on single nucleotide polymorphisms in the *HMG-CoA reductase* gene, Swerdlow *et al*.[87] demonstrated that reduced HMG-CoA reductase activity results in a slightly increased risk of type 2 diabetes (rs17238484-G allele odds ratio per allele 1.02, 95% confidence interval 1.00–1.05; rs12916 allele 1.06, 1.03–1.09). Therefore, it appears that an increased risk of type 2 diabetes is at least partially conferred by HMG-CoA reductase activity itself and is thus an on-target effect of statins. More recently, an observational study has suggested that transmembrane cholesterol transport is linked to the development of type 2 diabetes mellitus[88]. In a cross-sectional analysis in the Netherlands, the prevalence of type 2 diabetes in patients with familial hypercholesterolemia was significantly lower than among unaffected relatives[88]. LDL receptor-negative mutation carriers had the lowest prevalence followed by LDL-receptor deficient mutation carriers, apolipoprotein B mutation carriers, and unaffected relatives. Although these cross-sectional data are susceptible to survival bias, they suggest that higher LDL receptor activity may promote the development of type 2 diabetes mellitus.

6.2. HDL lipoproteins

Low HDL cholesterol levels were independently associated with heart failure incidence after adjustment for interim myocardial infarction and clinical covariates in

Framingham Heart Study participants free of coronary heart disease at baseline[69]. Earlier cross-sectional studies had demonstrated that post-infarct ejection fraction is lower in patients with low HDL cholesterol levels[77, 78]. Low HDL cholesterol levels and low levels of apolipoprotein (apo) A-I, the main apo of HDL, indicate an unfavorable prognosis in patients with heart failure independent of the aetiology[89, 90].

The observed relationship between HDL and heart failure in epidemiological studies might reflect residual confounding. Low HDL may be an integrated biomarker of adverse metabolic processes such as abnormal metabolism of triglyceride rich lipoproteins, insulin resistance, and ongoing tissue inflammation[91]. Crosstalk between inflammatory processes and metabolic dysregulation may accelerate the development of heart failure[92].

Human *apo A-I* gene transfer inhibits the development of diabetic cardiomyopathy in rats[93]. We have shown that HDL raising gene transfer raises both capillary density and relative vascularity in the myocardium of hypercholesterolemic mice and improves diastolic function in these mice as indicated by an increased peak rate of isovolumetric relaxation[94]. Furthermore, we have recently demonstrated that selective HDL raising human *apo A-I* gene transfer increases survival, reduces infarct expansion, attenuates left ventricular dilatation, and enhances systolic and diastolic cardiac function post-myocardial infarction in mice[95]. Molecular effects of HDL on the myocardium have been recently reviewed in detail[96]. Taken together, these experimental intervention studies corroborate the view that HDL-targeted therapies might be beneficial in prevention and/or treatment of heart failure.

CONCLUSION

The daily turnover of ATP in the human heart (13 mol or more than 6 kg) is many times higher than the weight of the heart[18]. Metabolic homeostasis requires a fine-tuning of cardiac metabolism of different substrates. Notwithstanding a retro control of fatty acid and glucose utilization, the heart functions best when it oxidizes both substrates simultaneously[19]. Mismatch between the uptake and oxidation of long-chain fatty acids in the myocardium induces lipotoxicity characterized by the accumulation of triglycerides, diacylglycerols, ceramides, and other lipids. Myocardial lipotoxicity may result in cardiomyocyte apoptosis, interstitial fibrosis, and cardiac dysfunction, and may promote insulin resistance[36]. Although lipotoxic heart disease is most evident in subjects with obesity and in patients with diabetes, epidemiological studies support a potential direct impact of fatty acids on the development of heart failure independent on any effect on coronary heart disease. Specifically, plasma levels of specific saturated fatty acids (C14:0 and C16:0) and of long-chain monounsaturated fatty acids (C22:1, C24:1) are positively associated with incidence of congestive heart failure[54, 63]. In contrast, intake of marine omega-3 polyunsaturated fatty acids (C20:5, *n*−3 and C22:6, *n*−3) lowered the risk of heart failure[65]. The relation between the intake of omega-6 polyunsaturated fatty acids and heart failure is unclear.

High non-HDL cholesterol levels are independently associated with heart failure incidence after adjustment for interim myocardial infarction and clinical covariates in Framingham Heart Study participants free of coronary heart disease at baseline[69]. Statins may be beneficial when started before the development of heart failure[79] but a clinically significant effect of initiating statin therapy in patients with established heart failure is insufficiently or not corroborated by randomized trials[80, 81]. A role

of HDL in myocardial biology and heart failure is supported by observational studies[69] and experimental human *apo A-I* gene transfer studies[93-95].

FUTURE PERSPECTIVE

The incidence and prevalence of heart failure are increasing[3]. Heart failure is the cardiovascular disease of this century in Europe and in the United States. As the population ages, heart failure will continue to be a growing public health problem. Future directions are summarized in Table 1.

The molecular basis of lipotoxic heart disease, specifically which lipid species are causing cardiac dysfunction, is insufficiently known. Lipotoxic heart disease and its relationship with cardiac cellular metabolism will remain an area of very intensive investigation. Lipidomics studies are required for quantification of individual ceramide species, diacylglycerol species, and species of other lipid classes to increase our insights into myocardial lipotoxicity.

Approximately 50% of patients with clinical features of chronic heart failure have heart failure with reduced ejection fraction (HFrEF) and 50% suffer from heart failure with preserved ejection fraction (HFpEF)[97]. HFpEF is a complex clinical syndrome that is characterized by classical heart failure symptoms with increased left ventricular filling pressure and preserved left ventricular ejection fraction. It is usually defined as heart failure with an ejection fraction equal to or greater than 50%. This heart failure subtype disproportionately affects women and the elderly and is commonly associated with other cardiovascular comorbidities, such as hypertension, obesity, and diabetes. Most clinical heart failure trials have been focused on patients with HFrEF. Inhibition of the renin-angiotensin-aldosterone and sympathetic nervous systems improves survival and decreases hospitalizations in patients with HFrEF. In contrast to these advances in treatment of patients with HFrEF, drug strategies with strong evidence in HFrEF have proved unsuccessful in HFpEF and the mortality in patients with HFpEF has remained unchanged. Since diastolic abnormalities constitute part of the

pathophysiology of HFpEF, HDL-targeted therapies might be beneficial in prevention and/or treatment of this particular type of heart failure.

The effectiveness of statins in patients with heart failure will remain an area of investigation. In the Swedish Heart Failure Registry, statins were associated with improved outcomes in patients with HFrEF, specifically in the presence of ischemic heart disease[98]. These real world data contrast with previous randomized controlled trials[80, 81]. Additional randomized controlled trials with more generalized inclusion or focused on ischemic heart disease may be warranted. Based on the analysis of a Japanese chronic heart failure registry, statin use was associated with improved mortality rates in HFpEF patients, mainly attributable to reductions in sudden death and noncardiovascular death[99]. Therefore, randomized controlled trials evaluating statins in patients with HFpEF are certainly warranted.

Consumption of flaxseed is a potential dietary intervention that might reduce heart failure incidence. Flaxseed contains α-linolenic acid (C18:3, *n*−3), lignans, and fiber. The Flaxseed for Peripheral Arterial Disease (FlaxPAD) Trial was a double-blinded randomized trial, in which patients with peripheral arterial disease, of whom 75% had hypertension, were randomized to 30 g of milled flaxseed or placebo daily for 6 months[100]. In the active treatment group, significant reductions of systolic (- 10 mm Hg) and diastolic blood pressure (-7 mm Hg) were observed. The antihypertensive effect was achieved selectively in hypertensive patients. Plasma α-linolenic acid (C18:3, *n*−3) increased with ingestion of flaxseed and was inversely associated with blood pressure. This effect may have been mediated via an inhibition of soluble epoxide hydrolase, which altered oxylipin concentrations[101].

In a randomized sample from the PREDIMED trial, a reduction of N-terminal pro-brain natriuretic peptide, a biomarker for heart failure, was observed in individuals at

high risk of cardiovascular disease who improved their diet toward a traditional Mediterranean diet compared with those assigned to a low-fat diet[102]. The critical question whether the effect of a traditional Mediterranean diet supplemented with extra-virgin olive oil or with mixed nuts reduces the incidence of heart failure should be addressed in a statistically sufficiently powered clinical trial in an elderly population at high risk for heart failure.

EXECUTIVE SUMMARY

• Epidemiological perspective on heart failure

*As the population ages, heart failure will continue to be a growing public health problem.

• Diet and prevention of coronary heart disease

* The PREDIMED study is a landmark trial in primary prevention.

* The interactions between various nutrients is the real biological source of protection rather than any small change in specific nutrients.

• Lipids as fuel for the heart

* The Randle cycle, also known as the glucose fatty-acid cycle, describes the reciprocal relationship between fatty acid and glucose metabolism. The mechanical efficiency of the heart is lower when fatty acids are oxidized.

• Cardiac lipotoxicity

* Mismatch between the uptake and oxidation of long-chain fatty acids in the myocardium induces lipotoxicity characterized by the accumulation of triglycerides, diacylglycerols, ceramides, and other lipids.

* Lipotoxicity may result in cardiomyocyte apoptosis, interstitial fibrosis, and cardiac dysfunction, and may promote insulin resistance.

• Impact of fatty acids on heart failure

* Plasma levels of specific saturated fatty acids (C14:0 and C16:0) and of long-chain monounsaturated fatty acids (C22:1, C24:1) are positively associated with incidence of congestive heart failure.

* In contrast, intake of marine omega-3 polyunsaturated fatty acids (C20:5, *n*−3 and C22:6, *n*−3) lowered the risk of heart failure.

* The relation between the intake of omega-6 polyunsaturated fatty acids and heart failure is unclear.

• Impact of lipoprotein levels on heart failure

* High non-HDL cholesterol levels and low HDL cholesterol levels are independently associated with heart failure incidence after adjustment for interim myocardial infarction and clinical covariates.

* The effect of statins in patients with established heart failure (HFrEF and HFpEF) remains controversial and is an area of active investigation.

* HDL-targeted therapies might be beneficial for prevention and/or treatment of heart failure.

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REFERENCES

1. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe: epidemiological update. *Eur Heart J* 34(39), 3028-3034 (2013).
2. Nichols M, Townsend N, Scarborough P, Rayner M. Trends in age-specific coronary heart disease mortality in the European Union over three decades: 1980-2009. *Eur Heart J* 34(39), 3017-3027 (2013).
3. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 35(42), 2950-2959 (2014).
4. Roger VL, Weston SA, Redfield MM *et al*. Trends in heart failure incidence and survival in a community-based population. *Jama* 292(3), 344-350 (2004).
5. Yancy WS, Jr., Westman EC, French PA, Califf RM. Diets and clinical coronary events: the truth is out there. *Circulation* 107(1), 10-16 (2003).
6. Weinberg SL. The diet-heart hypothesis: a critique. *J Am Coll Cardiol* 43(5), 731-733 (2004).
7. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *Jama* 251(3), 365-374 (1984).
8. Keys A, Menotti A, Karvonen MJ *et al*. The diet and 15-year death rate in the seven countries study. *Am J Epidemiol* 124(6), 903-915 (1986).
9. Keys A. Mediterranean diet and public health: personal reflections. *Am J Clin Nutr* 61(6 Suppl), 1321S-1323S (1995).
10. De Lorgeril M, Salen P, Martin JL *et al*. Effect of a mediterranean type of diet on the rate of cardiovascular complications in patients with coronary artery disease. Insights into the cardioprotective effect of certain nutriments. *J Am Coll Cardiol* 28(5), 1103-1108 (1996).
11. Estruch R, Ros E, Salas-Salvado J *et al*. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 368(14), 1279-1290 (2013).
12. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 348(26), 2599-2608 (2003).
13. De Lorgeril M. Mediterranean diet and cardiovascular disease: historical perspective and latest evidence. *Curr Atheroscler Rep* 15(12), 370 (2013).
14. Balaban RS. Maintenance of the metabolic homeostasis of the heart: developing a systems analysis approach. *Ann N Y Acad Sci* 1080, 140-153 (2006).
15. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 90(1), 207-258 (2010).
16. Opie LH. Metabolism of the heart in health and disease. I. *Am Heart J* 76(5), 685-698 (1968).
17. Opie LH. Metabolism of the heart in health and disease. II. *Am Heart J* 77(1), 100-122 contd (1969).
18. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation* 116(4), 434-448 (2007).
19. Taegtmeyer H. Metabolism--the lost child of cardiology. *J Am Coll Cardiol* 36(4), 1386-1388 (2000).
20. Wisneski JA, Gertz EW, Neese RA, Mayr M. Myocardial metabolism of free fatty acids. Studies with 14C-labeled substrates in humans. *J Clin Invest* 79(2), 359-366 (1987).

21. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schonekess BO. Regulation of fatty acid oxidation in the mammalian heart in health and disease. *Biochim Biophys Acta* 1213(3), 263-276 (1994).
22. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 85(3), 1093-1129 (2005).
23. Nielsen TS, Jessen N, Jorgensen JO, Moller N, Lund S. Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. *J Mol Endocrinol* 52(3), R199-222 (2014).
24. Niu YG, Hauton D, Evans RD. Utilization of triacylglycerol-rich lipoproteins by the working rat heart: routes of uptake and metabolic fates. *J Physiol* 558(Pt 1), 225-237 (2004).
25. Goldberg IJ, Trent CM, Schulze PC. Lipid metabolism and toxicity in the heart. *Cell Metab* 15(6), 805-812 (2012).
26. Takahashi S, Sakai J, Fujino T *et al*. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. *J Atheroscler Thromb* 11(4), 200-208 (2004).
27. Razani B, Zhang H, Schulze PC *et al*. Fatty acid synthase modulates homeostatic responses to myocardial stress. *J Biol Chem* 286(35), 30949-30961 (2011).
28. Garland PB, Randle PJ, Newsholme EA. Citrate as an Intermediary in the Inhibition of Phosphofructokinase in Rat Heart Muscle by Fatty Acids, Ketone Bodies, Pyruvate, Diabetes, and Starvation. *Nature* 200, 169-170 (1963).
29. Knaapen P, Germans T, Knuuti J *et al*. Myocardial energetics and efficiency: current status of the noninvasive approach. *Circulation* 115(7), 918-927 (2007).
30. Bing RJ, Hammond MM, Et Al. The measurement of coronary blood flow, oxygen consumption, and efficiency of the left ventricle in man. *Am Heart J* 38(1), 1-24 (1949).
31. Suga H. Ventricular energetics. *Physiol Rev* 70(2), 247-277 (1990).
32. Ten Velden GH, Elzinga G, Westerhof N. Left ventricular energetics. Heat loss and temperature distribution of canine myocardium. *Circ Res* 50(1), 63-73 (1982).
33. Hinkle PC. P/O ratios of mitochondrial oxidative phosphorylation. *Biochim Biophys Acta* 1706(1-2), 1-11 (2005).
34. Mjos OD. Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. *J Clin Invest* 50(7), 1386-1389 (1971).
35. Simonsen S, Kjekshus JK. The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man. *Circulation* 58(3 Pt 1), 484-491 (1978).
36. Holland WL, Knotts TA, Chavez JA, Wang LP, Hoehn KL, Summers SA. Lipid mediators of insulin resistance. *Nutr Rev* 65(6 Pt 2), S39-46 (2007).
37. Saddik M, Lopaschuk GD. Myocardial triglyceride turnover and contribution to energy substrate utilization in isolated working rat hearts. *J Biol Chem* 266(13), 8162-8170 (1991).
38. Strawford A, Antelo F, Christiansen M, Hellerstein MK. Adipose tissue triglyceride turnover, de novo lipogenesis, and cell proliferation in humans measured with 2H2O. *Am J Physiol Endocrinol Metab* 286(4), E577-588 (2004).

39. Liu L, Shi X, Bharadwaj KG *et al*. DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity. *J Biol Chem* 284(52), 36312-36323 (2009).
40. Ghafourifar P, Klein SD, Schucht O *et al*. Ceramide induces cytochrome c release from isolated mitochondria. Importance of mitochondrial redox state. *J Biol Chem* 274(10), 6080-6084 (1999).
41. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 51(7), 2005-2011 (2002).
42. Bayeva M, Sawicki KT, Ardehali H. Taking diabetes to heart--deregulation of myocardial lipid metabolism in diabetic cardiomyopathy. *J Am Heart Assoc* 2(6), e000433 (2013).
43. Ferre P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes* 53 Suppl 1, S43-50 (2004).
44. Hopkins TA, Sugden MC, Holness MJ, Kozak R, Dyck JR, Lopaschuk GD. Control of cardiac pyruvate dehydrogenase activity in peroxisome proliferator-activated receptor-alpha transgenic mice. *Am J Physiol Heart Circ Physiol* 285(1), H270-276 (2003).
45. Chiu HC, Kovacs A, Ford DA *et al*. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest* 107(7), 813-822 (2001).
46. Chiu HC, Kovacs A, Blanton RM *et al*. Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. *Circ Res* 96(2), 225-233 (2005).
47. Finck BN, Lehman JJ, Leone TC *et al*. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. *J Clin Invest* 109(1), 121-130 (2002).
48. Kienesberger PC, Pulinilkunnil T, Sung MM *et al*. Myocardial ATGL overexpression decreases the reliance on fatty acid oxidation and protects against pressure overload-induced cardiac dysfunction. *Mol Cell Biol* 32(4), 740-750 (2012).
49. Pulinilkunnil T, Kienesberger PC, Nagendran J *et al*. Myocardial adipose triglyceride lipase overexpression protects diabetic mice from the development of lipotoxic cardiomyopathy. *Diabetes* 62(5), 1464-1477 (2013).
50. Bjorkegren J, Veniant M, Kim SK *et al*. Lipoprotein secretion and triglyceride stores in the heart. *J Biol Chem* 276(42), 38511-38517 (2001).
51. Bartels ED, Nielsen JM, Hellgren LI, Ploug T, Nielsen LB. Cardiac expression of microsomal triglyceride transfer protein is increased in obesity and serves to attenuate cardiac triglyceride accumulation. *PLoS One* 4(4), e5300 (2009).
52. Nielsen LB, Bartels ED, Bollano E. Overexpression of apolipoprotein B in the heart impedes cardiac triglyceride accumulation and development of cardiac dysfunction in diabetic mice. *J Biol Chem* 277(30), 27014-27020 (2002).
53. Yokoyama M, Yagyu H, Hu Y *et al*. Apolipoprotein B production reduces lipotoxic cardiomyopathy: studies in heart-specific lipoprotein lipase transgenic mouse. *J Biol Chem* 279(6), 4204-4211 (2004).
54. Yamagishi K, Nettleton JA, Folsom AR. Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J* 156(5), 965-974 (2008).

55. Russo SB, Baicu CF, Van Laer A *et al*. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. *J Clin Invest* 122(11), 3919-3930 (2012).
56. Chess DJ, Khairallah RJ, O'shea KM, Xu W, Stanley WC. A high-fat diet increases adiposity but maintains mitochondrial oxidative enzymes without affecting development of heart failure with pressure overload. *Am J Physiol Heart Circ Physiol* 297(5), H1585-1593 (2009).
57. Chess DJ, Lei B, Hoit BD, Azimzadeh AM, Stanley WC. Effects of a high saturated fat diet on cardiac hypertrophy and dysfunction in response to pressure overload. *J Card Fail* 14(1), 82-88 (2008).
58. Roine P, Uksila E, Teir H, Rapola J. Histopathological changes in rats and pigs fed rapeseed oil. *Z Ernährungswiss* 1(2), 118-124 (1960).
59. Schiefer B, Loew FM, Laxdal V *et al*. Morphologic effects of dietary plant and animal lipids rich in docosenoic acids on heart and skeletal muscle of cynomolgus monkeys. *Am J Pathol* 90(3), 551-564 (1978).
60. Bremer J, Norum KR. Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet. *J Lipid Res* 23(2), 243-256 (1982).
61. Van Vleet JF, Ferrans VJ. Myocardial diseases of animals. *Am J Pathol* 124(1), 98-178 (1986).
62. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr* 21, 193-230 (2001).
63. Imamura F, Lemaitre RN, King IB *et al*. Long-chain monounsaturated Fatty acids and incidence of congestive heart failure in 2 prospective cohorts. *Circulation* 127(14), 1512-1521, 1521e1511-1518 (2013).
64. Mozaffarian D, Bryson CL, Lemaitre RN, Burke GL, Siscovick DS. Fish intake and risk of incident heart failure. *J Am Coll Cardiol* 45(12), 2015-2021 (2005).
65. Djousse L, Akinkuolie AO, Wu JH, Ding EL, Gaziano JM. Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis. *Clin Nutr* 31(6), 846-853 (2012).
66. Lemaitre RN, Sitlani C, Song X *et al*. Circulating and dietary alpha-linolenic acid and incidence of congestive heart failure in older adults: the Cardiovascular Health Study. *Am J Clin Nutr* 96(2), 269-274 (2012).
67. Petrone AB, Weir N, Hanson NQ *et al*. Omega-6 fatty acids and risk of heart failure in the Physicians' Health Study. *Am J Clin Nutr* 97(1), 66-71 (2013).
68. Kannel WB, Mcgee DL. Diabetes and cardiovascular disease. The Framingham study. *Jama* 241(19), 2035-2038 (1979).
69. Velagaleti RS, Massaro J, Vasan RS, Robins SJ, Kannel WB, Levy D. Relations of lipid concentrations to heart failure incidence: the Framingham Heart Study. *Circulation* 120(23), 2345-2351 (2009).
70. Kromhout D, Menotti A, Bloemberg B *et al*. Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. *Prev Med* 24(3), 308-315 (1995).
71. Talini E, Di Bello V, Bianchi C *et al*. Early impairment of left ventricular function in hypercholesterolemia and its reversibility after short term treatment with rosuvastatin A preliminary echocardiographic study. *Atherosclerosis* 197(1), 346-354 (2008).

72. Liu YB, Wu CC, Lu LS *et al*. Sympathetic nerve sprouting, electrical remodeling, and increased vulnerability to ventricular fibrillation in hypercholesterolemic rabbits. *Circ Res* 92(10), 1145-1152 (2003).
73. Rubinstein J, Pelosi A, Vedre A, Kotaru P, Abela GS. Hypercholesterolemia and myocardial function evaluated via tissue doppler imaging. *Cardiovasc Ultrasound* 7, 56 (2009).
74. Huang Y, Walker KE, Hanley F, Narula J, Houser SR, Tulenko TN. Cardiac systolic and diastolic dysfunction after a cholesterol-rich diet. *Circulation* 109(1), 97-102 (2004).
75. Luo TY, Su MJ, Yang YF *et al*. Effect of hypercholesterolemia on myocardial function in New Zealand white rabbits. *J Biomed Sci* 11(6), 829-837 (2004).
76. Van Craeyveld E, Jacobs F, Gordts SC, De Geest B. Low-density lipoprotein receptor gene transfer in hypercholesterolemic mice improves cardiac function after myocardial infarction. *Gene Ther* 19(8), 860-871 (2012).
77. Wang TD, Wu CC, Chen WJ *et al*. Dyslipidemias have a detrimental effect on left ventricular systolic function in patients with a first acute myocardial infarction. *Am J Cardiol* 81(5), 531-537 (1998).
78. Kempen HJ, Van Gent CM, Buytenhek R, Buis B. Association of cholesterol concentrations in low-density lipoprotein, high-density lipoprotein, and high-density lipoprotein subfractions, and of apolipoproteins AI and AII, with coronary stenosis and left ventricular function. *J Lab Clin Med* 109(1), 19-26 (1987).
79. Kjekshus J, Pedersen TR, Olsson AG, Faergeman O, Pyorala K. The effects of simvastatin on the incidence of heart failure in patients with coronary heart disease. *J Card Fail* 3(4), 249-254 (1997).
80. Kjekshus J, Apetrei E, Barrios V *et al*. Rosuvastatin in older patients with systolic heart failure. *N Engl J Med* 357(22), 2248-2261 (2007).
81. Tavazzi L, Maggioni AP, Marchioli R *et al*. Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* 372(9645), 1231-1239 (2008).
82. Moosmann B, Behl C. Selenoprotein synthesis and side-effects of statins. *Lancet* 363(9412), 892-894 (2004).
83. Loscalzo J. Keshan disease, selenium deficiency, and the selenoproteome. *N Engl J Med* 370(18), 1756-1760 (2014).
84. Felker GM. Coenzyme Q10 and statins in heart failure: the dog that didn't bark. *J Am Coll Cardiol* 56(15), 1205-1206 (2010).
85. Molyneux SL, Florkowski CM, George PM *et al*. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. *J Am Coll Cardiol* 52(18), 1435-1441 (2008).
86. Mortensen SA, Rosenfeldt F, Kumar A *et al*. The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. *JACC Heart Fail* 2(6), 641-649 (2014).
87. Swerdlow DI, Preiss D, Kuchenbaecker KB *et al*. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet* 385(9965), 351-361 (2015).
88. Besseling J, Kastelein JJ, Defesche JC, Hutten BA, Hovingh GK. Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. *Jama* 313(10), 1029-1036 (2015).

89. Mehra MR, Uber PA, Lavie CJ, Milani RV, Park MH, Ventura HO. High-density lipoprotein cholesterol levels and prognosis in advanced heart failure. *J Heart Lung Transplant* 28(9), 876-880 (2009).
90. Iwaoka M, Obata JE, Abe M *et al*. Association of low serum levels of apolipoprotein A-I with adverse outcomes in patients with nonischemic heart failure. *J Card Fail* 13(4), 247-253 (2007).
91. Gordts SC, Singh N, Muthuramu I, De Geest B. Pleiotropic effects of HDL: towards new therapeutic areas for HDL-targeted interventions. *Curr Mol Med* 14(4), 481-503 (2014).
92. Palomer X, Salvado L, Barroso E, Vazquez-Carrera M. An overview of the crosstalk between inflammatory processes and metabolic dysregulation during diabetic cardiomyopathy. *Int J Cardiol* 168(4), 3160-3172 (2013).
93. Van Linthout S, Spillmann F, Riad A *et al*. Human apolipoprotein A-I gene transfer reduces the development of experimental diabetic cardiomyopathy. *Circulation* 117(12), 1563-1573 (2008).
94. Gordts SC, Van Craeyveld E, Muthuramu I, Singh N, Jacobs F, De Geest B. Lipid Lowering and HDL Raising Gene Transfer Increase Endothelial Progenitor Cells, Enhance Myocardial Vascularity, and Improve Diastolic Function. *PLoS One* 7(10), e46849 (2012).
95. Gordts SC, Muthuramu I, Nefyodova E, Jacobs F, Van Craeyveld E, De Geest B. Beneficial effects of selective HDL-raising gene transfer on survival, cardiac remodelling and cardiac function after myocardial infarction in mice. *Gene Ther* 20(11), 1053-1061 (2013).
96. Van Linthout S, Frias M, Singh N, De Geest B. Therapeutic Potential of HDL in Cardioprotection and Tissue Repair. *Handb Exp Pharmacol* 224, 527-565 (2015).
97. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med* 355(3), 251-259 (2006).
98. Alehagen U, Benson L, Edner M, Dahlstrom U, Lund LH. Association between use of statins and outcomes in heart failure with reduced ejection fraction: prospective propensity score matched cohort study of 21 864 patients in the Swedish heart failure registry. *Circ Heart Fail* 8(2), 252-260 (2015).
99. Nochioka K, Sakata Y, Miyata S *et al*. Prognostic impact of statin use in patients with heart failure and preserved ejection fraction. *Circ J* 79(3), 574-582 (2015).
100. Rodriguez-Leyva D, Weighell W, Edel AL *et al*. Potent antihypertensive action of dietary flaxseed in hypertensive patients. *Hypertension* 62(6), 1081-1089 (2013).
101. Caligiuri SP, Aukema HM, Ravandi A, Guzman R, Dibrov E, Pierce GN. Flaxseed consumption reduces blood pressure in patients with hypertension by altering circulating oxylipins via an alpha-linolenic acid-induced inhibition of soluble epoxide hydrolase. *Hypertension* 64(1), 53-59 (2014).
102. Fito M, Estruch R, Salas-Salvado J *et al*. Effect of the Mediterranean diet on heart failure biomarkers: a randomized sample from the PREDIMED trial. *Eur J Heart Fail* 16(5), 543-550 (2014).

REFERENCE ANNOTATIONS

Reference 3

* This paper represents an authoritative and recent overview of cardiovascular epidemiology in Europe.

Reference 11

** This paper presents the results of the landmark primary prevention PREDIMED trial.

Reference 42

** This review is an excellent discussion of fatty acid metabolism and alterations of lipid homeostasis in the diabetic heart.

Reference 63

** This prospective epidemiological study represents the rediscovery of the importance of long-chain monounsaturated fatty acids in cardiac metabolism and development of heart failure.

Reference 65

* This paper is an authoritative meta-analysis demonstrating the effect of dietary intake of C20:5, *n*−3 and C22:6, *n*−3 on the incidence of congestive heart failure.

Reference 76

** This experimental murine intervention study unequivocally demonstrates the impact of plasma cholesterol on the development of ischemic heart failure independent of any effects on the coronary circulation.

Reference 96

** This review contains a detailed description of molecular effects of HDL on the myocardium.

LEGEND TO THE FIGURE

Figure 1. **Schematic overview of fatty acid ß-oxidation in cardiomyocytes and its reciprocal relationship with glucose metabolism.** Fatty acids enter the cardiomyocyte either by passive diffusion or via protein carrier-mediated pathways including CD36 (fatty acid translocase) and fatty acid transport protein (FATP) 1. In the cytosol, fatty acids are esterified to fatty-acyl-CoA by fatty-acyl-CoA synthase. Fatty-acyl-CoA is then esterified to triglycerides (TG) or converted to fatty-acyl-carnitine via carnitine palmitoyltransferase I (CPT1), which is an integral membrane protein that associates with the outer mitochondrial membrane. Carnitine-acylcarnitine translocase (CACT) is responsible for transporting fatty-acyl-carnitine across the inner mitochondrial membrane. In the mitochondrial matrix, fatty-acyl-carnitine is converted to fatty-acyl-CoA by carnitine palmitoyltransferase II (CPT2). The fatty-acyl-CoA enters the cycle of ß-oxidation pathway involving formation of trans-2-enoyl-CoA, 3-hydroxyacyl-CoA, beta-ketoacyl-CoA, and back to fatty-acyl-CoA via the enzymes acyl-CoA dehydrogenase, enoyl-CoA hydratase 1, 3-hydroxyacyl-CoA dehydrogenase and beta-ketoacyl-CoA thiolase, respectively. Acetyl-CoA produced in the ß-oxidation pathway enters the tricarboxylic acid cycle (TCA cycle) (Krebs cycle, citric acid cycle), which results in the production of $FADH_2$ and NADH. In certain conditions, fatty-acyl-CoA can be cleaved into fatty acid anions (FA^-) and CoA via the enzyme mitochondrial thioesterase (MTE). The FA^- can then exit the mitochondrial matrix via an uncoupling protein (UCP). The electrons from cellular dehydrogenases in the ß-oxidation pathway and TCA cycle are transferred horizontally in the electron transport chain in the inner mitochondrial membrane accompanied by a vertical transfer of dehydrogenase derived protons from the mitochondrial matrix into the intermembrane space creating an electrochemical

proton gradient across the inner mitochondrial membrane. This gradient is the driving force in formation of ATP from ADP through the FO/F1-ATP synthase complex. UCPs in the inner mitochondrial membrane can dissipate the proton gradient so that free energy to drive ATP synthesis is lost.

Glucose can enter the cardiomyocyte via GLUT4 or GLUT1 transporters. Pyruvate decarboxylation is the key irreversible step in carbohydrate oxidation and is catalyzed by pyruvate dehydrogenase, which is located in the mitochondrial matrix. Increased generation of acetyl-CoA derived from fatty acid β-oxidation decreases pyruvate dehydrogenase activity via the activation of pyruvate dehydrogenase kinase and the subsequent phosphorylation and inhibition of pyruvate dehydrogenase. Both an increased acetyl CoA/CoA ratio and an increased NADH/NAD^+ ratio result in activation of pyruvate dehydrogenase kinase.